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APPLICATION FOR UNITED STATES LETTERS PATENT

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Title: OCULAR THERAPY WITH REDUCED OCULAR NEOVASCULARIZATION

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SPECIFICATION

OCULAR THERAPY WITH REDUCED OCULAR NEOVASCULARIZATION

FIELD OF THE INVENTION

The invention is directed to methods and compositions that control neovascularization or regress existing vascularization, and additionally treat ocular disease.

BACKGROUND

Ocular neovascularization is the pathologic ingrowth of blood vessels in the cornea, retina, or choroid. The new vessels can cause reduced vision or loss of vision due to bleeding and subsequent scarring, fibrosis, etc.

Blood vessel growth or formation can be due to diverse events. These include hypoxia (e.g., in diabetes), inflammatory responses (e.g., blepharitis), microbial infection (e.g., keratitis), physical insult (e.g., improper use

of contact lenses), chemical insult (e.g., toxins), pharmacologic agents, or other factors (e.g., graft rejection). More specifically, an inflammatory response may follow corneal transplant. Ocular microbial infections include but are not limited to trachoma, viral interstitial keratitis, and keratoconjunctivitis. Corneal insult may be due to contact with acidic or alkaline solutions, trauma, improper hygiene and/or compliance with contact lens use, such as extended wear lenses, or chemical agents such as silver nitrate. Other factors leading to ocular neovascularization include mechanical irritation of the limbal sulcus, corneal hypoxia, epithelial cell erosion or hypertrophy. In dry eye disease (conjunctiva sicca), the dehydrated conditions cause sloughing off of the epithelium, resulting in new vessel formation.

Methods of treating ocular neovascularization have met with limited success. These include treatment of the underlying condition, if possible; topical corticosteroid application for gross and active vascularization; diathermy of large feeding vessels and corneal laser photocoagulation for treatment of superficial vascularization of the cornea with infiltration of granulation tissue (pannus); and even limbal grafting for severe chemical injuries and limbal epithelium loss.

Other methods and compositions which reduce or prevent ocular neovascularization, and which may treat an ocular pathology, are desirable.

SUMMARY OF THE INVENTION

Compositions and methods useful to reduce ocular neovascularization (new blood vessels in the cornea, retina, conjunctiva, and/or choroid) are disclosed. The compositions contain at least one of a steroid,

doxycycline, and/or heparin, and may additionally contain an antimicrobial, an anti-prostaglandin, and/or a metalloproteinase inhibitor. The steroid may be triamcinolone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, fluorometholone, rimexolone, medrysone, lotoprednol etabonate, 11-desoxycortisol, and/or anacortave acetate. In this embodiment, the compositions are in pharmaceutically acceptable formulations for topical ocular application, ocular injection, or ocular implantation, and may be contained in liposomes or slow release capsules.

In one embodiment, the composition comprises doxycycline at a concentration from about 0.01 µg/ml to about 30 mg/ml and a steroid at a concentration from about 0.1 mg/ml to about 40 mg/ml. In another embodiment, the composition comprises heparin in a concentration from about 0.01 µg/ml to about 30 mg/ml and a steroid at a concentration from about 0.1 mg/ml to about 40 mg/ml. In another embodiment, the composition comprises doxycycline in a concentration from about 0.01 µg/ml to about 30 mg/ml and heparin in a concentration from about 0.01 µg to about 30 mg/ml.

One embodiment is a method for reducing ocular neovascularization by administering a composition containing a steroid, such as those previously listed, and doxycycline and/or heparin in a therapeutically effective amount for a duration sufficient to reduce ocular neovascularization. The composition may also contain an anti-prostaglandin, a metalloproteinase inhibitor, or an antimicrobial, including doxycycline or another antimicrobial, as

previously described. The method achieves a reduction or regression of vascularization throughout the treatment duration with a non-toxic composition.

One embodiment of the method uses a composition without a steroid. Because steroids are known to increase intraocular pressure (glaucoma), this embodiment is beneficial for patients with glaucoma or at risk for glaucoma, and for patients after glaucoma filtering surgery.

In another embodiment, the invention method administers the steroid, heparin, and doxycycline in a cyclic tumor treatment regimen to reduce blood vessel growth and proliferation at a tumor site. In this embodiment, the agents are systemically administered along with standard tumor therapies, so that the agents are rotated, thereby inhibiting blood vessel proliferation throughout the treatment cycle.

These and other advantages will be apparent in light of the following figures and detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a photograph of a rat eye to which a saline control was administered.

FIG. 2 is a photograph of a rat eye to which a composition of one embodiment of the invention was administered.

DETAILED DESCRIPTION

An ocular treatment regimen and composition is disclosed that limits, reduces, slows the rate of, or prevents ocular neovascularization, and/or that causes regression of existing new blood vessels, generally referred to as

reduced neovascularization, although the term encompasses any degree of inhibition by any method and also encompasses any degree of regression of existing vessels. In various embodiments, doses and formulations of the inventive composition are administered to a patient in addition to, or as treatments for, an ocular pathology. The inventive methods and compositions may desirably inhibit ocular neovascularization that occurs from any event, for example, due to ocular disease, hypoxia, trauma, physical or chemical insult, etc.

Ocular neovascularizations can be superficial or deep and may lead to loss of optical transparency through stromal hemorrhage, scarring, lipid deposition, etc. Neovascularizations may occur in any area of the eye, such as the cornea, retina, conjunctiva, or choroid. The presence of new vessels may result in an increased intraocular pressure, termed neovascular glaucoma or ocular hypertension. The new vessels may lead to hemorrhage and fibrosis, and result in structural damage to the eye with subsequent decreased visual acuity. For example, corneal burns result in the formation of new vessels that can decrease vision as they infiltrate and penetrate the cornea. In corneal transplants, new blood vessels from the limbus penetrate the cornea and may result in rejection of the engrafted tissues. Thus, control or prevention of new vessels to any extent is desirable, although greater inhibition is more desirable and total inhibition of new vessels is most desirable.

The inventive composition comprises a pharmaceutically acceptable formulation (that is, containing buffers and excipients as known to one skilled in the art) of any one of the following agents used alone or in any

combination: one or more steroids, doxycycline, or heparin. In embodiments, one or more of the following may also be included: anti-angiogenesis agents, anti-prostaglandins, metalloproteinase inhibitors, and antimicrobial agents other than doxycycline, such as macrolide antibiotics. In one embodiment, the composition comprises a steroid in a concentration and dose to reduce neovascularization in the absence of doxycycline and in the absence of heparin. In another embodiment, the composition comprises doxycycline in a concentration and dose to reduce neovascularization in the absence of a steroid and in the absence of heparin. In another embodiment, the composition comprises heparin in a concentration and dose to reduce neovascularization in the absence of one or more steroids and doxycycline. In another embodiment, a formulation of a steroid and doxycycline is ocularly administered in a concentration and dose to reduce neovascularization. In another embodiment, a formulation of a steroid and heparin is ocularly administered in a concentration and dose to reduce neovascularization. In another embodiment, a formulation of doxycycline and heparin is ocularly administered in a concentration and dose to reduce neovascularization. In another embodiment, a formulation of a steroid, doxycycline, and heparin is ocularly administered in a concentration and dose to reduce neovascularization.

In one embodiment, the inventive composition includes steroids. Steroids are administered for ocular pathologies such as uveitis, diabetic retinopathy, idiopathic juxtafoveal telangiectasias, macular edema secondary to diabetes mellitus, central retinal vein occlusion, pseudophakia, during

photodynamic therapy for age related macular degeneration, etc., and for intraoperative visualization of the posterior hyaloid, which also desirably inhibit ocular neovascularization.

An undesirable and serious side effect of ocular steroid therapy is increased intraocular pressure, termed glaucoma or ocular hypertension. For patients with glaucoma or predisposed to glaucoma, steroid therapy presents a risk for unacceptably high intraocular pressure, such that surgery may be required to lower the intraocular pressure. Such risks and benefits must be balanced in determining whether to treat the patient with triamcinolone or other steroids. The compositions and methods to predict patients at risk for glaucoma from steroid therapy, disclosed in co-pending U.S. Patent Application Serial No. 10/787,580, which is expressly incorporated by reference herein in its entirety, may desirably inhibit neovascularization.

Steroids for ocular administration include, but are not limited to, triamcinolone (Aristocort®; Kenalog®), betamethasone (Celestone®), budesonide, cortisone, dexamethasone (Decadron-LA®; Decadron® phosphate; Maxidex® and Tobradex® (Alcon)), hydrocortisone, methylprednisolone (Depo-Medrol®, Solu-Medrol®), prednisolone (prednisolone acetate, e.g., Pred Forte® (Allergan); Econopred and Econopred Plus® (Alcon); AK-Tate® (Akorn); Pred Mild® (Allergan); prednisone sodium phosphate (Inflamase Mild and Inflamase Forte® (Ciba); Metreton® (Schering); AK-Pred® (Akorn)), fluorometholone (fluorometholone acetate (Flarex® (Alcon); Eflone®), fluorometholone alcohol (FML® and FML-Mild®, (Allergan); FluorOP®)), rimexolone (Vexol® (Alcon)),

medrysone alcohol (HMS® (Allergan)); lotoprednol etabonate (Lotemax® and Alrex® (Bausch & Lomb), 11-desoxcortisol, and anacortave acetate (Alcon)). It will be appreciated that the above lists are representative only and are not exclusive.

The steroid concentration in the inventive composition ranges from about 0.1 mg/ml to about 40 mg/ml. The concentration used with a particular formulation may depend upon the particular steroid that is used. For example, triamcinolone acetonide (9α-fluoro-11β, 16α, 17, 21 tetrahydroxy-pregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone ($C_{24}H_{31}FO_6$)) Kenacort®, Kenalog® (Bristol-Myers Squibb, Princeton NJ) may be administered at a therapeutic dose in the range of about 4 mg to about 8 mg, for example, by intravitreous injection. It may also be administered intravitreally in a challenge dose ranging from about 50 μg to about 800 μg to determine patients at risk for developing a steroid-induced increase in intraocular pressure when these patients are administered a therapeutic dose of triamcinolone by intravitreal injection, as disclosed in the co-pending U.S. Patent Application Serial No. 10/787,580. In comparison, anacortave acetate, a steroid with less potential to cause an increase in intraocular pressure than triamcinolone but not used inside the eye, may be administered at dose of about 0.5 mg to about 30 mg.

Heparin is a heterogeneous group of straight-chain anionic mucopolysaccharides, termed glycosaminoglycans, having anticoagulant activity. The primary sugars are α-L-iduronic acid 2-sulfate, 2-deoxy-2-sulfamino-α-D-glucose 6-sulfate, ß-D-glucuronic acid, 2-acetamido-2-deoxy-α-D-glucose, and α-

L- iduronic acid. These sugars are present in different amounts and are joined by glycosidic linkages, forming polymers of varying sizes. Heparin is strongly acidic because of its content of covalently linked sulfate and carboxylic acid groups. In heparin sodium, the acidic protons of the sulfates are partially replaced by sodium ions. In one embodiment of the invention, low molecular weight heparin is used. Low molecular weight heparin is derived from standard heparin through either chemical or enzymatic depolymerization, and is commercially available. Standard heparin has a molecular weight of about 5,000 daltons to about 30,000 daltons, while low molecular weight heparin has a molecular weight of about 1,000 daltons to about 10,000 daltons. Compared to standard heparin, low molecular weight heparin binds less strongly to protein, has enhanced bioavailability, interacts less with platelets and yields a predictable dose response and dose-dependent plasma levels, and produces less bleeding for a given antithrombotic effect. Low molecular weight heparin may be heparin sulfate, a lower-sulfated, higher-acetylated form of heparin. All of these are commercially available (e.g., Sigma Aldrich, St. Louis MO).

Doxycycline (4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1, 11-dioxo-2-naphthacenecarboxamide monohydrate, $C_{22}H_{24}N_2O_8 \cdot H_2O$) is a broad spectrum antibiotic in the class of tetracycline antibiotics. It is commercially available.

Other antibiotics may be added to the inventive composition. For example, macrolide antibiotics include tacrolimus, cyclosporine, sirolimus, everolimus, ascomycin, erythromycin, azithromycin, clarithromycin, clindamycin,

lincomycin, dirithromycin, josamycin, spiramycin, diacetyl-midecamycin, tylosin, roxithromycin, ABT-773, telithromycin, leucomycins, and lincosamide. Other antibiotics include, but are not limited to, aminoglycosides (e.g., streptomycin, amikacin, gentamicin, tobramycin), cephalosporins (e.g., beta lactams including penicillin), tetracyclines, acyclorvir, amantadine, polymyxin B, amphtotericin B, amoxicillin, ampicillin, atovaquone, azithromycin, azithromycin, bacitracin, cefazolin, cefepime, cefotaxime, cefotetan, cefpodoxime, ceftazidime, ceftizoxime, ceftriaxone, cefuroxime, cephalexin, chloramphenicol, clotimazole, ciprofloxacin, clarithromycin, clindamycin, dapsone, dicloxacillin, fluconazole, foscarnet, ganciclovir, gatifloxacin, griseofulvin, isoniazid, itraconazole, ketoconazole, metronidazole, nafcillin, neomycin, nitrofurantoin, nystatin, pentamidine, rifampin, rifamycin, valacyclovir, vancomycin, etc. The indications, effective doses, formulations, contraindications, vendors, etc. of these antibiotics are known to one skilled in the art.

Anti-prostaglandins include indomethacin, ketorolac tromethamine 0.5% ((±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1) (ACULAR® Allegan, Irvine CA), OCUFEN® (flurbiprofen sodium 0.03%), meclofenamate, fluorbiprofen, and compounds in the pyrrolo-pyrrole group of non-steroidal anti-inflammatory drugs.

Any of the agents may be administered in any formulation, such as a slow release formulation, a carrier formulation such as microspheres, microcapsules, liposomes, etc., an intravenous solution or suspension, or an intraocular injection, as known to one skilled in the art. A time-release drug

delivery system may be administered intraocularly to result in sustained release of the agent over a period of time. The formulation may be in the form of a vehicle, such as a micro- or macro-capsule or matrix of biocompatible polymers such as polycaprolactone, polyglycolic acid, polylactic acid, polyanhydrides, polylactide-co-glycolides, polyamino acids, polyethylene oxide, acrylic terminated polyethylene oxide, polyamides, polyethylenes, polyacrylonitriles, polyphosphazenes, poly(ortho esters), sucrose acetate isobutyrate (SAIB), and other polymers such as those disclosed in U.S. Patent Nos. 6,667,371; 6,613,355; 6,596,296; 6,413,536; 5,968,543; 4,079,038; 4,093,709; 4,131,648; 4,138,344; 4,180,646; 4,304,767; 4,946,931, each of which is expressly incorporated by reference herein in its entirety, or lipids that may be formulated as microspheres or liposomes. A microscopic or macroscopic formulation may be administered through a needle, or may be implanted by suturing within the eye, for example, within the lens capsule. Delayed or extended release properties may be provided through various formulations of the vehicle (coated or uncoated microsphere, coated or uncoated capsule, lipid or polymer components, unilamellar or multilamellar structure, and combinations of the above, etc.). The formulation and loading of microspheres, microcapsules, liposomes, etc. and their ocular implantation are standard techniques known by one skilled in the art, for example, the use a ganciclovir sustained-release implant to treat cytomegalovirus retinitis, disclosed in Vitreoretinal Surgical Techniques, Peyman et al., Eds. (Martin Dunitz, London 2001, chapter 45); Handbook of Pharmaceutical Controlled Release Technology, Wise, Ed. (Marcel

Dekker, New York 2000), the relevant sections of which are incorporated by reference herein in their entirety. For example, a sustained release intraocular implant may be inserted through the pars plana for implantation in the vitreous cavity. An intraocular injection may be into the vitreous (intravitreal), or under the conjunctiva (subconjunctival), or behind the eye (retrobulbar), or under the Capsule of Tenon (sub-Tenon), and may be in a depot form. Other intraocular routes of administration and injection sites and forms are also contemplated and are within the scope of the invention.

The route and form of administration may be any method known to one skilled in the art, and as previously described. In one embodiment, the formulation is intraocularly injected, for example, into the vitreous. The steroid(s) and agent(s) may be administered as a mixture, an admixture, in the same formulation, in separate formulations, etc. The agent(s) may be administered topically, or may be injected into the eye, or one agent may be administered topically and the other agent(s) may be injected. For example, in preparation for injection, topical alcaine was applied to the ocular surface, followed by 5% povidone iodine. A cotton-tipped applicator soaked in 4% lidocaine was then applied to the injection site, which is 4.0 mm posterior to the limbus in phakic eyes and 3.5 mm posterior to the limbus in pseudophakic eyes. A 27-gauge needle was used for injection at the superior pars plana. Indirect ophthalmoscopy confirmed proper intravitreal placement of the suspension.

The effect of a particular steroid, hydrocortisone 21-phosphate, with

low molecular weight heparin in inhibiting neovascularization in the cornea has been reported (Lepri et al., J. Ocular Pharmacol. 10, 273, 1994, which is expressly incorporated by reference herein it its entirety). There was about a 60% reduction in the amount and length of blood vessels when hydrocortisone and low molecular weight heparin were administered to rats at a dose of two drops per eye, four times daily, for six days. However, this evaluation did not address the issues of toxicity or the effect on visual acuity in a living patient. It also did not address the effect of intraocular pressure. Further, it did not evaluate other commonly prescribed steroids such as triamcinolone, which is a frequently prescribed steroid for ocular pathologies in human patients, nor did it include doxycycline or the agents alone.

The agents are administered in an amount or at a concentration that does not result in intraocular toxicity. For example, low molecular weight heparin may be administered in a concentration ranging from about 0.01 µg/ml to about 30 mg/ml. In one embodiment, the concentration of low molecular weight heparin ranges from about 0.5 mg/ml to about 20 mg/ml (for example, administration of 0.1 ml of a 100 mg/ml formulation of low molecular weight heparin). In various embodiments, the concentration may be about 0.5 mg/ml to about 2.5 mg/ml, about 1 mg/ml to about 5 mg/ml, about 5 mg/ml to about 10 mg/ml, or about 5 mg/ml to about 30 mg/ml.

Doxycycline may be administered in a concentration ranging from about 0.01 µg/ml to about 30 mg/ml. In another embodiment, doxycycline concentrations range from about 0.05 mg/ml to about 1 mg/ml. In another

embodiment, doxycycline concentrations range from about 0.05 mg/ml to about 10 mg./ml. In another embodiment, doxycycline concentrations range from about 1 mg/ml to about 20 mg/ml. Besides its anti-angiogenic effect, doxycycline could reduce the incidence of endophthalmitis, which occurs in about 0.5% of eyes in which a steroid is administered.

A steroid may be administered in a concentration ranging from about 0.1 mg/ml to about 40 mg/ml. In another embodiment, steroid concentrations range from about 1 mg/ml to about 20 mg/ml. In another embodiment, steroid concentrations range from about 20 mg/ml to about 30 mg/ml. In another embodiment, steroid concentrations range from about 20 mg/ml to about 40 mg/ml.

Macrolide antibiotics may be administered in a concentration ranging from about 20 μg/ml to about 200 μg/ml (about 0.002%$^{w/v}$ to about 0.02%$^{w/v}$). Compositions and doses of macrolide antibiotics are described in co-pending U.S. Patent Application Serial Nos. 10/667,161 and 10/752,124, each of which is expressly incorporated by reference herein in its entirety. In one embodiment, a concentration of macrolide antibiotic and/or mycophenolic acid in a pharmaceutically acceptable topically administered solution may range from about 0.5%$^{w/v}$ to about 10%$^{w/v}$. In another embodiment, a concentration of macrolide antibiotic and/or mycophenolic acid in a pharmaceutically acceptable topically administered solution may range from about 3%$^{w/v}$ to about 5%$^{w/v}$. In another embodiment, a concentration of macrolide antibiotic and/or mycophenolic acid in a pharmaceutically acceptable topically administered

solution may range from about 1%$^{w/v}$ to about 3%$^{w/v}$. In another embodiment, a concentration of macrolide antibiotic and/or mycophenolic acid in a pharmaceutically acceptable topically administered solution may range from about 3%$^{w/v}$ to about 10%$^{w/v}$. In another embodiment, a concentration of macrolide antibiotic and/or mycophenolic acid may range from about 0.1% to about 10% in a topical ocular formulation for treating diabetic retinopathy, age related macular degeneration, or retinitis pigmentosa. In another embodiment, concentrations of macrolide antibiotic and/or mycophenolic acid up to about 2%, up to about 5%, up to about 10%, or exceeding 10% are formulated for topical administration when the compound(s) is bound to a matrix or polymer which slowly releases the compound(s) over time while not exceeding an intraocular concentration of 40 μg/ml.

Anti-prostaglandins, also termed prostaglandin antagonists, may be administered in a concentration sufficient to result in a prostaglandin-inhibitory effect. For example, ACUALR® may be administered at a concentration ranging from about 0.003%$^{w/w}$ to about 0.3%$^{w/w}$. In one embodiment, the concentration of ACULAR® is about 0.03%$^{w/w}$. As another example, OCUFEN® (flurbiprofen sodium) 0.03% may be administered at a concentration ranging from about 0.003%$^{w/w}$ to about 0.3%$^{w/w}$.

The inventive method and composition reduces or eliminates the risk of ocular neovascularization, which may occlude the cornea or other structures, leading to reduced vision. A possible mechanism for the beneficial effect of heparin in reducing vessel growth and proliferation is its polyanionic

structure, which readily binds to polycationic angiogenic factors. Angiogenic factors with heparin bound to them have reduced biological activity, and therefore do not promote new vessel growth. A possible mechanism for the beneficial effect of doxycycline in reducing vessel growth and proliferation is its inhibition of metalloproteinases, which are zinc-dependent proteinase enzymes associated with the tumorigenic process. They are used in the angiogenic process as well as in tumor metastasis. In some human cancers a positive correlation has also been demonstrated between the intensity of new blood vessel growth (angiogenesis) and the likelihood of developing metastases. Thus, control of metalloproteinase activity in these two different contexts has generated considerable interest as a possible therapeutic target. Inhibitors of metalloproteinases include naturally occurring proteins such as TIMP-1 that specifically inhibit matrix metalloproteinases, and synthetic metalloproteinase inhibitors such as Batimastat (BB-94) and marimastat (BB-2516) which potently and specifically inhibit metalloproteinase production. These inhibitors degrade the extracellular matrix, promoting tumor invasion and metastasis, but also regulate host defense mechanisms and normal cell function. Selective inhibition is expected to inhibit reactions leading to vascularization in the inventive compositions and methods. Such metalloproteinase inhibitors are also included in the invention. A possible mechanism for the beneficial effect of macrolide antibiotics are their anti-inflammatory effect.

In another embodiment, administration of the inventive formulation may cause regression of existing new vessels. Vessel regression may occur in

addition to, or in place of, prevention of further vessel growth or proliferation. As will be appreciated, the cumulative effects may be important in managing diseases such as diabetes, where control of the complicating factors of the disease is as important as control of the underlying pathology to maintain a patient's quality of life.

In another embodiment of the invention, a steroid is intraocularly administered with one or more of the above-described agents. The Example demonstrates the additional beneficial effect when steroids, administered to treat various ocular diseases, are combined with other agents such as doxycycline or low molecular weight heparin, and may be used to achieve significant inhibition of neovascularization for the duration of treatment.

In another embodiment, a non-steroid containing composition that reduces neovascularization is administered. Such a composition eliminates the steroid-induced increased intraocular pressure that may result. As described, in patients at risk for glaucoma or with pre-existing glaucoma, control of neovascularization without the concomitant risks of increase intraocular pressure, are beneficial.

For example, surgery to relieve the increased intraocular pressure that occurs with glaucoma (glaucoma filtering surgery) may itself result in neovascularization. Filtering surgery is performed on patients with increased intraocular pressure when drugs and laser surgery do not adequately lower intraocular pressure. A trabeculectomy or sclerostomy is performed to create a small hole through which fluid drains from the eye, thereby reducing intraocular

pressure. After time, however, the surgically created hole begins to close as the body attempts to self-heal the wound. As the hole closes, the intraocular pressure begins to rise. The inventive composition is able to reduce this process so that the hole remains open allowing fluid to drain. The composition, topically administered to the eye, inhibits neovascularization and cell proliferation in this region. The composition also desirably inhibits the ocular scarring in glaucoma filtering blebs. Blebs are small blisters that are usually located on the upper surface of the eye, as a result of intraocular fluid flowing through the surgically created hole

For these patients, doxycycline, low molecular weight heparin, or a combination of doxycycline and low molecular weight heparin may be administered. In various embodiments, the agent(s) is/are administered topically or are injected. For topical administration, concentrations up to 30 mg/ml of doxycycline and/or heparin, are administered, and any dose may be effective depending upon the particular patient, the underlying disease and its severity, the dosing frequency, etc., as known to one skilled in the art. Sample concentrations include, but are not limited to, about 0.5 mg/ml to about 2.5 mg/ml, about 1 mg/ml to about 5 mg/ml, about 5 mg/ml to about 10 mg/ml, about 10 mg/ml to about 15 mg/ml, about 15 mg/ml up to 30 mg/ml, etc. For injection, a concentration less than about 1 mg/ml may be injected, and any amount may be effective depending upon the factors previously described. Sample concentrations include, but are not limited to, about 5 µg/ml to about 50 µg/ml; about 25 µg/ml to about 100 µg/ml; about 100 µg/ml to about 200 µg/ml; about 200 µg/ml to about

500 µg/ml; about 500 µg/ml to about 750 µg/ml; about 500 µg/ml up to 1 mg/ml; etc. Formulations may be mixtures, admixtures, extended release formulations, liposomes, microcapsules, or any of the previously described embodiments.

In another embodiment, doxycycline and heparin may be administered to a patient following corneal surgery (e.g., LASIK® surgery, photorefractive keratectomy (PRK), or other corneal procedures) to reduce ocular irritation. Doxycycline, or other antibiotics, and heparin inhibit collagenase and metalloproteinase enzymes, which otherwise result in deposits that damage and cloud the cornea. In this embodiment, a supratherapeutic concentration of doxycycline and heparin may be used. For example, doxycycline and/or heparin at a concentration up to about 40 mg/ml may be used.

In another embodiment, an anti-prostaglandin agent may be administered with doxycycline and/or heparin. The anti-prostaglandin may be administered at the doses and by the methods previously described, and include indomethacin, ketorolac tromethamine 0.5% ((±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1) (ACULAR® Allegan, Irvine CA), OCUFEN® (flurbiprofen sodium 0.03% (Allergan) (sodium (±)-2-(2-fluoro-4-biphenylyl)-propionate dihydrate), meclofenamate, fluorbiprofen, and compounds in the pyrrolo-pyrrole group of non-steroidal anti-inflammatory drugs (NSAIDs).

EXAMPLE 1

Artificial corneal burns were induced in rat eyes to determine the effects of doxycycline, steroids, and low molecular weight heparin, alone and in

combinations, on corneal neovascularization. More specifically, topical administration of doxycycline, low molecular weight heparin, and triamcinolone were administered twice a day to rats in which corneal burns had been artificially induced by application of silver nitrate (70%) and potassium nitrate (30%).

The presence of new vessels (neovascularization) and the extent of new vessel formation was assessed by split lamp photography and histology. Inhibition of vessel proliferation was evaluated by measuring vessel progression from the outer cornea (corneal limbus) into the cornea. A numerical rating system was used to quantitate the degree of inhibition (+, ++, and +++ inhibition), with "+ inhibition" indicating inhibition one-third of the distance from the limbus of the cornea to the center; "++ inhibition" indicating inhibition two-thirds of the distance from the limbus to the center; "+++ inhibition" indicating complete inhibition of vessels between the limbus and the center; and the designation "$\pm$ inhibition" indicating an intermediate degree of inhibition (e.g, less than +, ++, or +++) . As previously described, it will be appreciated that any reduction of new vessel proliferation and/or regression of existing vessels is therapeutic, and that complete inhibition and/or regression is not required, and also that reduction includes regression of existing vessels.

Full vascularization was seen after one week of saline administration (control), as seen in FIG. 1. Any of the above agents alone, when topically applied to affected corneas, did not completely inhibit neovascularization. For example, corneas treated with topically applied doxycycline at a concentration of about 1 mg/ml to about 20 mg/ml showed

+ inhibition of neovascularization compared to controls. Corneas treated with topically applied low molecular weight heparin at a concentration of about 10 mg/ml showed + inhibition of neovascularization compared to controls. Corneas treated with topically applied triamcinolone at a concentration of about 4 mg/ml showed ++ inhibition of neovascularization.

In contrast, when a composition of doxycycline (about 20 mg/ml) and triamcinolone (4 mg/ml) was topically applied to the affected cornea twice a day, there was +++ inhibition of neovascularization; that is, no neovascularization was evident. The +++ inhibition of new vessel growth was seen at one week after treatment, and the same +++ inhibition was maintained at three weeks, as shown in FIG. 2.

When a composition of low molecular weight heparin (about 10 mg/ml) and triamcinolone was topically applied to the affected cornea twice a day, there was +++ inhibition of neovascularization after one week compared to the control eye. After three weeks, the inhibition of neovascularization was minimally diminished (++±) so that neovascularization inhibition was slightly less than the doxycycline and triamcinolone composition applied, but there was still significant inhibition.

When a composition of low molecular weight heparin (about 1 mg/ml) and doxycycline (about 20 mg/ml) was topically applied to the affected cornea twice a day, neovascularization was also inhibited after one week but to a lesser extent (++ to +++) compared to administration with either doxycycline and triamcinolone, or low molecular weight heparin and triamcinolone. After three

weeks, there was still complete inhibition of neovascularization with doxycycline and low molecular weight heparin compared to controls. Neovascularization was not observed for the treatment duration.

EXAMPLE 2

The ability of the inventive composition to cause regression of existing vessels was demonstrated. Neovascularization was induced over three days by topical application of a silver nitrate solution, as described in Example 1, to thirty-two rat eyes. Vascularization was allowed to proceed midway from the limbus to the cornea (days 1, 2, and 3).

On day 4, one dose (15 µl) of one of the following treatments was administered to the affected eyes (eight eyes per group): saline (control); a composition of triamcinolone (40 mg/ml) and low molecular weight heparin (10 mg/ml); a composition of doxycycline (20 mg/ml) and low molecular weight heparin (10 mg/ml); or a composition of doxycycline (20 mg/ml) and triamcinolone (40 mg/ml). The same treatment regimen was repeated on each eye on both of days 5 and 6.

Eyes were examined on day 6. All of the control eyes showed vascular progression, in that the eyes were fully vascularized and no inhibition of vascularization occurred. That is, vascularization extended from the limbus to the cornea.

In contrast, all the treated eyes, regardless of the treatment composition, showed regression of vascularization. Eyes treated with triamcinolone and low molecular weight heparin showed ++ to +++ reduced

vascularization. Eyes treated with doxycycline and low molecular weight heparin showed + to ++ reduced vascularization. Eyes treated with doxycycline and triamcinolone showed ++ reduced vascularization.

In another embodiment, the inventive compositions may be used in an overall cyclic treatment regimen for tumors occurring anywhere in the body. In this embodiment, the initial therapy (stage 1) is selected among those presently available: either chemotherapy (e.g., gene therapy, antineoplastic drugs, etc.) or one or more of the following non-chemotherapeutic treatments: radiation therapy (e.g, x-rays, gamma rays, ß rays, etc.); phototherapy (e.g., photodynamic therapy, photosensitizers); or thermal therapy (e.g., thermal coagulation, hyperthermia, cryotherapy).

Immediately following this initial treatment event, therapy using the inventive compositions is initiated in a rotational cycle. That is, a steroid, doxycycline, and heparin is administered over the course of one cycle, but these agents are administered at different stages in the cycle. Each of the agents is administered systemically (e.g., intravenously, orally, etc.) at their highest non-toxic concentration, as known to one skilled in the art. For example, steroids are administered at doses ranging from about 100 mg/ml to about 200 mg/ml. The use of a cyclic rotational administration of each of these vessel-inhibiting agents causes vessel damage at different times and through different processes, thereby maximizing the overall damage to the vessels and inhibiting blood supply to the tumor while conventional tumor therapy occurs (e.g., chemotherapy, radiation therapy, etc.).

The inventive cyclic therapy is initiated by systemic administration of a steroid, followed by systemic administration of a composition containing the same or another steroid and doxycycline (stage 2). For example intravenous administration of methylprednisolone (Solu-Medrol®) can be followed by oral administration of prednisone and doxycycline. Stage 2 lasts from about one to about two weeks. Stage 3 follows stage 2, during which a composition containing doxycycline and heparin is administered. Chemotherapeutic drugs may also be administered in stage 3. Stage 3 lasts from about one to about two weeks. Stage 4 follows stage 3, during which a composition containing doxycycline, anti-prostaglandins, and macrolide antibiotics are administered. Stage 4 lasts from about one to about two weeks and completes the first treatment cycle, which lasts from about one to about two months.

If additional therapy is required (determined by tumor size, the presence of absence of tumor markers, etc.), further cycle(s) of treatment are initiated. These further cycles may start either with stage 1 and proceed through stages 2, 3, and 4, or may start with stage 2 directly from stage 4 and bypass stage 1. It will be appreciated that any of the agents described herein may be used in any of stages 2, 3, or 4. For example, anti-prostaglandins may be used in place of low molecular weight heparin in stage 3; low molecular weight heparin may be used in place of doxycycline in either or both of stages 2 and/or 3, etc.

Other variations or embodiments of the invention will also be apparent to one of ordinary skill in the art from the above descriptions. Thus, the

forgoing embodiments are not to be construed as limiting the scope of this invention.

What is claimed is: